



# Carbon flow through energycane agroecosystems established post-intensive agriculture

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## Abstract

As part of an integrated energy and climate system, biomass production for bioenergy based on the tropical perennial C<sub>4</sub> grass energycane can both offset fossil fuels and store soil carbon (C). We measured energycane yields, root biomass, soil C pools, and soil C stocks in a 4 year field trial and modeled C flow from plants to soils in the surface layer of no-till energycane planted after more than a century of intensive sugarcane agriculture. Aboveground yields ranged from 16.7 to 19.0 Mg C/ha over the 4 year trial. Although total C stocks did not significantly differ in the surface layer (approx. 0–20 cm) during the study, C in free and occluded light fractions decreased, whereas C in the mineral-rich dense fraction increased over 4 years. Belowground system inputs, estimated from measurements and informed by convergence in the final soil fraction model, were set to 2.5 Mg C ha<sup>-1</sup> year<sup>-1</sup>. With this input value, we estimated that surface soils retained photosynthetically fixed C predominantly within the mineral-associated organic matter pool for a mean and median transit time of 177 and 110 years, respectively. Although we did not model C flow to deep soil layers (approx. 0–100 cm), observed C accumulation (11.4 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and root growth down to 120 cm suggest that soil processes and resulting C sequestration at the surface are likely to persist deeper into the soil profile. Energycane, as a strong candidate for climate change mitigation and land degradation remediation, showed high biomass yields and allocation of resources to roots, with sequestered soil C expected to persist for over a century.

## KEYWORDS

agroecosystem, bioenergy, carbon, climate change energycane, land degradation, tropical perennial grasses

## 1 | INTRODUCTION

Soil carbon (C) sequestration during biomass production for bioenergy can compound the expected climate benefit derived only from offsetting fossil fuel use, thereby improving its viability as a renewable energy pathway (Whitaker et al., 2018). Land is a critical resource, and land degradation is pervasive globally (IPCC, 2019) with degraded and abandoned agricultural areas increasingly targeted for land-based CO<sub>2</sub> removal. Carefully managed grass-based biofuel crops not only draw down C from the atmosphere and offset fossil fuel use, but also improve ecosystem diversity, health, and resilience (Anderson-Teixeira et al., 2012; DeLucia, 2016). For more than a century, the pineapple and sugarcane industry dominated agricultural landscapes in Hawaii. However, over the last 40 years, low market prices and high labor costs drove a sharp decline in production and subsequent increase in abandoned prime agricultural lands (Agricultural Land Use, 2015). Future integrated food, energy, and climate systems in Hawaii, as in many other tropical and subtropical regions worldwide, will require the restoration of degraded landscapes previously under intensive cultivation (Dallimer & Stringer, 2018; Morgan, Youkhana, Turn, Ogoshi, & Garcia-Pérez, 2019). Therefore, it is important to understand and accurately project the potential climate benefits of tropical C<sub>4</sub> perennial grass agroecosystems cultivated on degraded lands.

Particularly in the tropics, the flow of C from plant inputs to soil in perennial grass agroecosystems remains poorly represented in agronomic process-based models (Meki, Kiniry, Behrman, Pawlowski, & Crow, 2014; Wells et al., 2017). Ratoon harvest is a common form of no-till management in sugarcane and other perennial grasses that leaves the lowest part of the plant and living roots intact during harvest (Ball-Coelho, Sampaio, Tiessen, & Stewart, 1992; Matsuoka & Stolf, 2012). When the soil physical environment and ecosystem remain undisturbed, resulting improvements in aggregation, rhizosphere development, and decreased soil C losses from erosion and respiration all contribute to soil C accumulation (Clifton-Brown, Breuer, & Jones, 2007; Pawlowski et al., 2017). Roots are of particular importance to stabilized soil C (Rasse, Rumpel, & Dignac, 2005) and species differences in allocation belowground and root depth distribution can be a key factor in deep soil profile C sequestration (Poirier, Roumet, & Munson, 2018; Sumiyoshi et al., 2016). For example, genotype-specific root distribution determined soil C sequestration in a temperate *Miscanthus* study (Richter, Agostini, Redmile-Gordon, White, & Goulding, 2015).

High-yielding tropical perennial grasses such as sugarcane, energycane and napiergrass use C<sub>4</sub> photosynthesis to produce large amounts of aboveground and root biomass (Morgan et al., 2019). Aboveground biomass typically correlates with roots (Bolinder, Angers, Bélanger, Michaud, & Laverdière, 2002).

Work on yield and growth rates exists for bioenergy crops grown across a range of environments and islands in Hawaii, and many cultivars are selected for deep rooting characteristics related to improved drought tolerance, reduced water requirements, and erosion control (Youkhana et al., 2017). In a variety and species trial, Sumiyoshi et al. (2016) found that tropical perennial C<sub>4</sub> grasses with proportionately fewer dead roots following harvest showed faster regrowth. At the same time, cultivars with high root biomass C, high proportion of root death following harvest, low lignin concentration, and fast root decay rate (*k*) accumulated more soil C.

Soil C is a heterogeneous matrix comprised of multiple pools with varying degrees of stabilization due to complex processes that protect organic matter from microbial accessibility and subsequent decomposition (Blankinship et al., 2018). These processes include chemical recalcitrance (e.g., pyrogenic C), occlusion within aggregates, and organo-mineral binding. It is typically thought that no-till management leads to rapid accumulation of C within soil aggregates (Six, Elliott, Paustian, & Doran, 1998; Tisdall & Oades, 1982; Zheng et al., 2018), while longer-term C storage and saturation behavior is driven by more gradual accumulation in mineral-associated soil organic matter (Briedis et al., 2018; Stewart, Paustian, Conant, Plante, & Six, 2007). For example, aggregate formation and stability were affected by soil type in giant miscanthus in the midwestern United States, thereby reducing the protection of recent C inputs from losses in some cases (Tiemann & Grandy, 2015). In contrast, the dynamic transfer of fresh inputs from roots through aggregates and into organo-mineral stabilized pools drove the rapid soil C accumulation measured in tropical perennial C<sub>4</sub> grasses (Crow, Deem, Sierra, & Wells, 2018; Crow & Sierra, 2018). In another study, Plaza, Courtier-Murias, Fernández, Polo, and Simpson (2013) similarly found that organo-mineral interactions increased soil C by 16% under no-till compared to conventional management. Turnover time of mineral-associated organic matter is longer than that of aggregate protected and particulate organic matter (Torn, Trumbore, Chadwick, Vitousek, & Hendricks, 1997; von Lützow et al., 2008), and most mineral-associated OM derives from root inputs driving production of microbial byproducts (Cotrufo et al., 2015; Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013). Therefore, further quantifying the amount and fate of belowground C input is critical to understanding the full potential climate benefit of C sequestration in tropical perennial grass systems.

In this study, we measured aboveground yield, soil C stocks, and soil physical fractions over 4 years of an experimental trial for the tropical perennial C<sub>4</sub> grass energycane (*Saccharum officinarum* × *Saccharum robustum* cv. MOL-6081) cultivated following more than a century of intensive agriculture. A model of surface soil C dynamics was developed to describe belowground inputs and movement through multiple soil pools with varying cycling rates to better

understand the soil process resulting in high soil C accumulation rates measured following energycane cultivation. We also applied the final mathematical model to a previously reported model (Crow et al., 2018; Crow & Sierra, 2018) for a similar tropical perennial grass system for verification and comparison. The persistence of C in this and similar tropical grass systems appears to be driven by rapid transfer from inputs to mineral-associated organic matter pools and we expect inputs and transit times of C through the agroecosystem to affect the potential climate benefit of sequestration and therefore of the bioenergy system as a whole.

## 2 | MATERIALS AND METHODS

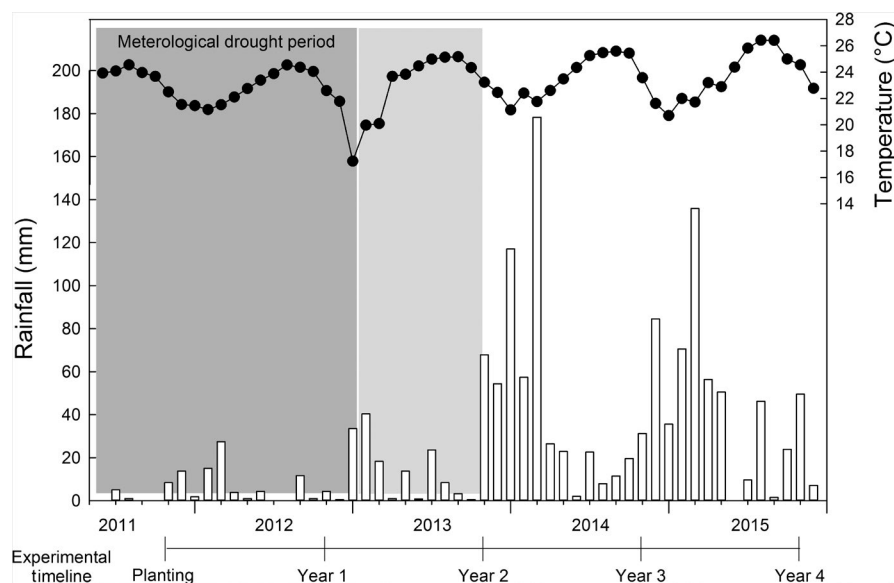
### 2.1 | Study site and experimental design

The field study was located on the island of Maui within the former sugarcane fields of Hawaiian Commercial and Sugar (HC&S). Commercial sugarcane production existed on Maui for over 125 years. At the time of the study, HC&S was the only remaining sugarcane plantation in Hawaii. Grown on a 2 year crop cycle, irrigated sugarcane at HC&S reached maturity after 24 months and was harvested following foliar glyphosate application, low-intensity burn, deep tillage, preparation tillage, and mechanized planting. In September 2011, the ratoon harvest, perennial grass field plots were established in a recently harvested sugarcane field (commercial field F718, 20.854, -156.466) that had been in an intensive cane-on-cane rotation for at least a century. However, in January 2016 HC&S announced a wholesale transition on their 14,000 ha plantation to diversified agriculture, including perennial grasses for forage, pasture, and bioenergy feedstock (Pawlowski, Meki, Kiniry, & Crow, 2018). The land was then sold to another owner before implementation of the

transition, the vast majority of land lies fallow at the time of writing.

The experimental plots were within a fine, kaolinitic, isohyperthermic, Aridic Haplustolls of the Ewa series at an elevation of 34 m above mean sea level. The climate is characterized by cool and persistent trade winds and mild temperatures, with a dry summer season from May through September and rainy winter season from October through April. Mean annual temperature is 26.7°C and mean annual precipitation is 88 mm (HC&S weather station data). The experiment ran from 2011 to 2015 and encompassed both drought and non-drought periods (Figure 1). Although actual evapotranspiration accounted for 75%–80% of the total water consumption plantation-wide (Osorio, Jeong, Bieger, & Arnold, 2014) and the HC&S sugarcane did not generally show signs of water stress (Anderson, Wang, Tirado-Corbalá, Zhang, & Ayars, 2015), growth responses to wet periods were often observed (M. Nakahata, HC&S, personal communication).

Prior to the initiation of the field trial, 10 baseline soil cores were collected randomly from the designated experimental area of F718. The profile was sampled at 20 cm depth increments to 160 cm using a standard wet core diamond-tipped drill bit with an internal diameter of 7 cm (Diamond Products Core Borer). A rotating hydraulic drill inserted the core barrel to ensure accurate depth measurements and minimize compaction. Soil samples were sieved at <2 mm and dried for 48 hr at 105°C. Subsamples were ground to pass through a 250 µm sieve for heterogeneity, weighed, and analyzed for elemental C and N. Flash combustion micro elemental analysis was used to determine C and N concentration (Costech ECS 4010 CHNSO Analyzer; Costech Analytical Technologies Inc.). All samples were analyzed in duplicate and averaged. Soil pH was measured in a 2:1 soil:water mixture and ranged from 7.0 to 7.8 throughout the profile.



**FIGURE 1** Mean monthly rainfall (bars) and temperature (black circles) measured from 2011 to 2015 at Hawaiian Commercial and Sugar commercial field 718. Extreme drought period is shown in dark grey and moderate drought period is shown in light grey. The experimental field study is shown with initial planting and annual harvests (Year 1–Year 4) indicated on the timeline

The field trial was a randomized complete block design with three replicates. Three species: sugarcane (*S. officinarum* cv. HA 65–7052), energycane (*S. officinarum* × *S. robustum* cv. MOL-6081) and napiergrass hybrid (*Pennisetum purpureum* × *Pennisetum glaucum* cv. banagrass), were selected for their high potential for perennial bioenergy production and yield performance based on data from previous evaluations at HC&S plantation. The ratoon harvest life cycles of the three crops are 2 years, 1 year, and 6 months, respectively. Each plot (15 × 11 m) consisted of four rows of grass, with two lines of stem cutting laid per row, the distances between rows and lines were 1.8 and 0.9 m, respectively. For all crops, 45 cm stem cuttings were planted end to end in 15 cm deep furrows in September 2011. Plants were drip irrigated as needed to prevent water stress. All plots received a total of 375 kg N/ha of fertilizer, based on HC&S sugarcane cultural practices, which was applied through the drip irrigation system that serviced the whole plantation. At ratoon, all grasses were cut at approximately 20 cm above the ground, leaving the belowground system intact and allowing for aboveground biomass to quickly return to preharvest levels. Though all crops were grown similarly, energycane was selected for further investigation of soil C dynamics in this study.

## 2.2 | Annual measurements of aboveground yield, soil C stocks, and surface C pools

The aboveground energycane yield was quantified annually for 4 years. At each harvest, aboveground material from a standard area (i.e., 9.1 m of the center four rows) was hand cut and weighed in the field. Ten stalks were randomly selected for dry weight determination at the field station and placed in a forced draft oven at 60°C until stable. A moisture factor was calculated as the ratio of dry:fresh weight, applied to the measured experimental area, and scaled up to Mg/ha of dry matter. In 2012 and 2013, the dried stalks were coarsely ground in a Wiley mill, then subsampled and finely ground to pass through a 250 µm sieve. All tissue samples were analyzed in duplicate for C and N concentration as described above and averaged. The C concentrations of tissue samples ranged from 44.9% to 45.2% and were averaged to obtain a single C concentration value used to calculate aboveground C yields.

Following each annual harvest, three deep soil cores were collected from each experimental plot for soil C stock determination. Soil cores were taken using hand augers, in 20 cm increments to 120 cm. Soil samples were passed through a 2 mm sieve and oven dried, homogenized, subsampled and finely ground to pass through a 250 µm sieve, and analyzed for C and N concentration as described above. Because a change in bulk density was reasonably expected over the course of the field trial, the equivalent soil mass (ESM) method (Crow et al., 2016; Wendt & Hauser, 2013) was used to determine

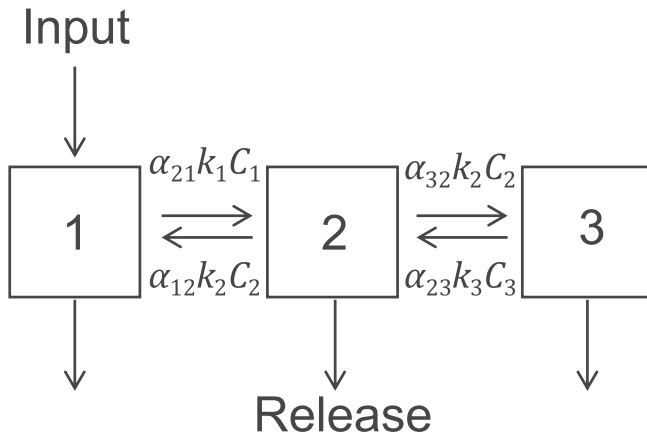
soil C stocks for the baseline and annual assessments. Carbon stocks for the surface soil (approx. 0–0 cm) and deep soil profile (approx. 0–100 cm) are reported. The three soil profiles collected from each experimental plot were averaged for a plot value, and the three plots' values from the field replicates were used to calculate the reported mean and standard error. The surface soil C stock is the ESM increment of 3,600 Mg/ha, which is approximately equal to a stock reported at 20 cm by the fixed depth method. The deep soil profile C stock is the ESM increment of 18,000 Mg/ha, which is approximately equal to a stock reported at 100 cm by the fixed depth method.

Soil from each of the 0–20 cm depth increments was physically fractionated to represent multiple soil C pools with varying rates of turnover. The method applied was according to Golchin, Oades, Skjemstad, and Clarke (1994): sodium polytungstate was used to isolate free light, occluded light, and dense fractions sequentially separated in a 1.8 g/ml solution. The free-light fraction, which represents fresh plant inputs like roots and litter, was separated from the soil through light agitation by hand followed by centrifugation (at 1,305 g for 10 min) and aspiration. To obtain the occluded light fraction, which represents C that has been physically protected by soil aggregation, the soil slurry was sonicated (400 J/ml total) to disrupt aggregates, and the released occluded C was captured by centrifugation and aspiration. The remainder of the soil was quantified as dense fraction. A more detailed description of the same procedure can be found in Crow et al. (2018). The recovered fraction weights and C concentrations were measured as described above and used to calculate the distribution of C among the pools. The proportionate distribution of C among pools was applied to the surface soil C stock described above to calculate each pool size for the baseline and annual assessments.

## 2.3 | Conceptual and mathematical model development

Conceptually, aboveground vegetation is associated with a belowground root system and some of those roots die upon ratoon harvest. Both live and dead roots contribute C inputs to the soil C system, but these are difficult to measure. The fate of C released from dead root turnover is complex; at least some is likely to enter the soil C system directly or via microbial biomass. Other implied inputs to soil C are those attributable to the live roots, including exudates and cell slough age. As a first-order estimate of belowground inputs, root biomass and depth distribution were determined at Year 1 and Year 2 using a destructive root sampling technique (Knoll, Anderson, Strickland, Hubbard, & Malik, 2012). Coarse live and dead roots >2 mm were collected from the sieve surface during soil processing described above. Any remaining identifiable roots were hand-picked from the soil that passed





**FIGURE 2** Conceptual diagram for the three-pool model expressed in Equation (1). Each pool has its own decomposition rate  $k_i$  and the coefficients  $\alpha_{ij}$  represent transfers among pools

through the sieve and were added to those >2 mm. All roots were dried at 65°C and weighed.

In 2012 and 2013, energycane root systems were excavated from pits opened using hand tools and 6 mm sieves at each plot. Roots from each pit were collected, quantified volumetrically from three depths: 0–40, 40–80 and 80–120 cm. Roots were washed and dried at 60°C until constant dry weight was achieved. The dried roots were coarsely ground in a Wiley mill, then subsampled and finely ground to pass through a 250  $\mu\text{m}$  sieve. All tissue samples were analyzed in duplicate for C and N concentration as described above and averaged. The C concentration values ranged from 40.9% to 42.7% and were averaged to obtain a single C concentration value (41.9%) that was applied to all root biomass values to calculate root C inputs.

To develop a mathematical model of the dynamic soil system, observations of soil C fractions over time and the estimated amount of belowground root inputs were used to derive a dynamic model that simulates future trajectories of total C stocks (Figure 2). Root C inputs were estimated in order to constrain variables as well as possible for 1 year of grass production. The model simulates the dynamic behavior of the free light, occluded light, and dense fractions, considering transfers and transformations of the organic matter and is similar in structure to the model used in a previous study on tropical perennial grasses (Crow et al., 2018; Crow & Sierra, 2018). The set of differential equations that describe the model are given by Equation (1).

$$\begin{aligned}\frac{dC_1}{dt} &= I + \alpha_{12}k_2C_2 - k_1C_1 \\ \frac{dC_2}{dt} &= \alpha_{21}k_1C_1 + \alpha_{23}k_3C_3 - k_2C_2 \\ \frac{dC_3}{dt} &= \alpha_{32}k_2C_2 - k_3C_3,\end{aligned}\quad (1)$$

where  $C_1$ ,  $C_2$  and  $C_3$  are the C stocks in the free light, occluded light, and dense fraction, respectively.  $I$  represents

plant inputs to soil; decomposition rates for each pool are represented as  $k_i$  and transfers among pools are represented by  $\alpha_{ij}$ .

A two-step procedure was used to obtain the parameters of the model using data from the density fractions and fixing C inputs according to the method mentioned previously. The optimization procedure consisted of first running the Nelder–Mead optimization algorithm to find the best set of parameters that minimizes the difference between predictions and observations. Then, the results from this optimization were used as priors in a Bayesian procedure that uses Markov chain Monte-Carlo methods to derive posterior distributions of the parameters and quantify uncertainty in model predictions (Soetaert & Petzoldt, 2010). R packages SoilR and FME were used to implement the model and the optimization (Sierra, Müller, & Trumbore, 2012; Soetaert & Petzoldt, 2010).

Determining the climate benefit of sequestration in terrestrial ecosystems requires knowledge of C inputs and the transit time (or, process rates) of those inputs through various ecosystem components. The compartmental model (Figure 2; Equation 1) underlying the computation represents inputs and process rates, which correspond to system aspects such as the fate of C inputs to ecosystem components, microbial controls, and inherent soil properties. The formulas described in Metzler and Sierra (2018) were applied to the optimized model obtained for the site to obtain the transit time distribution of C of the system and calculate mean and median ages. Age distributions are a strong indicator of C persistence in soil and transit time is indicative of how long C inputs remain before leaving the system (Sierra, Hoyt, He, & Trumbore, 2018). Means and medians of each of these parameters are important to understanding the system under study.

Uncertainty in the model parameter estimates was explored by systematically sampling input values at increments of 0.5 within the range of 2–3  $\text{Mg C ha}^{-1} \text{ year}^{-1}$ , outside of which the model failed. Mean parameter values are reported with a standard deviation derived from the uncertainty analysis. The modeling results from a related study on the island of Oahu (Crow et al., 2018; Crow & Sierra, 2018) were compared to predictions of the final model for energycane; particularly, with respect to how the different fractions are expected to behave over time. Results were compared in terms of total C stocks. The code that supports the findings of this study is available in the Supporting Information of this article.

## 2.4 | Statistical analysis

Differences in root C input by depth under energycane were analyzed using a linear mixed model (LMM) in R

(R Core Team, 2020) using the lme4 (Bates, Mächler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017) packages and associated linear mixed-effect regression (lmer) function where depth was a fixed factor and rep nested in year ( $n = 2$ ) was a random effect. For parsimony, all carbon stock data (i.e. aboveground biomass, surface soil, surface soil fractions, and the deep soil profile) collected over the 4 year trial were similarly analyzed using LMMs with year as a fixed factor and rep as a random effect. Because time is our only treatment, this statistical approach effectively implements a “repeated measures” interdependency effect from year to year comparisons. Post hoc Tukey's tests ( $p < .05$ ) were accomplished using the emmeans package (Length, 2020) in R.

### 3 | RESULTS

#### 3.1 | Annual aboveground yield, soil C stocks, and surface C pools

Mean aboveground yield ranged from 16.7 to 19.0 Mg C/ha and did not differ statistically year to year during the study period (Table 1). In the surface soil, total C stock (ranging from 66.8 to 77.4 Mg C/ha) also did not differ significantly from year to year. However, C in the light fractions decreased over time whereas C in the dense fraction increased. Free-light fraction decreased from  $6.3 \pm 0.9$  to  $2.7 \pm 0.4$  Mg C/ha from baseline to Year 4 respectively; occluded light fraction decreased from  $16.0 \pm 0.8$  to  $8.3 \pm 1.1$  Mg C/ha from baseline to Year 4, respectively. Deep soil C stock increased significantly from baseline ( $180.1 \pm 9.7$  Mg C/ha) to Year 2 ( $224.5 \pm 4.3$  Mg C/ha), dropped down in Year 3 ( $201.6 \pm 5.2$  Mg C/ha), then increased significantly compared to the baseline again in Year 4 ( $225.7 \pm 7.0$  Mg C/ha).

**TABLE 1** Measured C stocks (Mg C/ha) in biomass, surface layer soils, surface layer soil fractions, and the deep soil profile over the course of the 4-year trial. Values are mean  $\pm$  1 SE. Letters show significant difference by year within each C stock ( $p < .05$ )

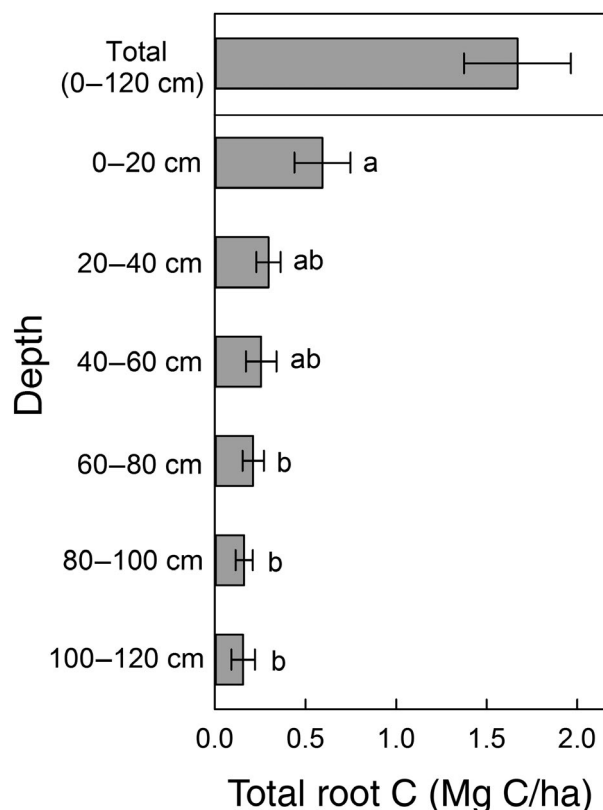
	Baseline	Year 1	Year 2	Year 3	Year 4
Aboveground yield	n/a	$16.7 \pm 1.7^a$	$18.2 \pm 2.7^a$	$19.0 \pm 2.1^a$	$17.3 \pm 1.1^a$
Surface soil					
Total	$67.3 \pm 4.3^a$	$70.2 \pm 1.5^a$	$75.3 \pm 0.6^a$	$66.8 \pm 2.4^a$	$77.4 \pm 4.0^a$
Free LF	$6.3 \pm 0.9^a$	$4.2 \pm 0.6^{ab}$	$4.9 \pm 0.9^{ab}$	$2.6 \pm 0.3^b$	$2.7 \pm 0.4^b$
Occl. LF	$16.0 \pm 0.8^a$	$13.4 \pm 1.7^{ab}$	$12.8 \pm 1.5^{ab}$	$8.8 \pm 1.2^b$	$8.3 \pm 1.1^b$
DF	$44.9 \pm 0.4^c$	$52.5 \pm 2.2^b$	$57.7 \pm 2.2^b$	$55.4 \pm 1.0^b$	$66.4 \pm 1.4^a$
Deep soil profile	$180.1 \pm 9.7^b$	$193.8 \pm 4.0^{ab}$	$224.5 \pm 4.3^a$	$201.6 \pm 5.2^{ab}$	$225.7 \pm 7.0^a$

Note: Surface layer = approx. 0–20 cm, deep soil profile = approx. 0–100 cm.

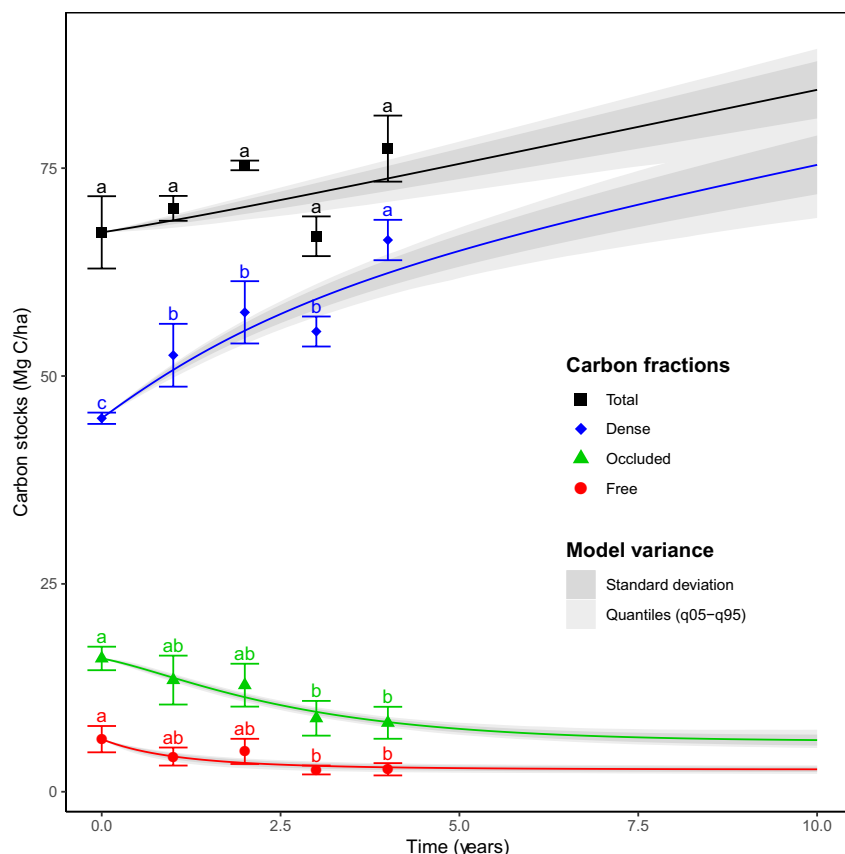
Abbreviations: DF, dense fraction; LF, light fraction; Occl., occluded.

#### 3.2 | Root biomass

Significant differences in root biomass C were not detected between Year 1 and Year 2, but depth contrasts were significant for surface (0–20 cm) and deeper depths (60–80, 80–100, and 100–120 cm; Figure 3). Total root biomass was  $1.7 \pm 0.3$  Mg C/ha and decreased from  $0.59 \pm 0.16$  to  $0.16 \pm 0.06$  Mg C/ha in the 0–20 to 100–120 cm depths, respectively.



**FIGURE 3** Total and incremental root biomass C ( $n = 6$ , including Year 1 and Year 2 data). Bars are means with standard errors. Means with different letters are significantly different ( $p < .05$ )



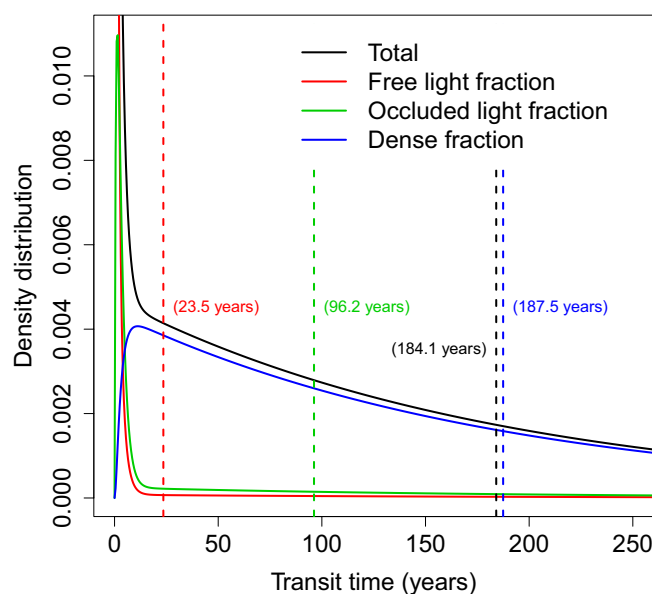
**FIGURE 4** Measured soil C stock in bulk soil and density fractions in the 0–20 cm depth increment and resulting dynamic soil C model projection. Based on the outcome of the sensitivity analysis, the C input value was set to  $2.5 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  for the simulation shown. Means with different letters were significantly different,  $p < .05$

**TABLE 2** Model parameter estimates. Input was varied from 2 to  $3 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  by 0.05 increments to estimate model sensitivity

	Input/decay rates ( $\text{Mg C ha}^{-1} \text{ year}^{-1}$ )				Transfer rates (%)			
	C input	$k_1$	$k_2$	$k_3$	$\alpha_{21}$	$\alpha_{12}$	$\alpha_{32}$	$\alpha_{23}$
Mean	2.50	1.20	0.55	0.010	89.8	15.3	80.4	55.3
SD	0.31	0.15	0.04	0.002	1.4	2.4	2.8	2.9

### 3.3 | Modeled C flow in energycane and comparative systems

The measured root biomass C ( $1.7 \text{ Mg C/ha}$ ) was set as the initial input parameter estimate and we obtained a relatively good agreement between our observations and final model predictions of total C stock and pool sizes (Figure 4). The model would not fit without a minimum input of  $2.0 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ , while over  $3.0 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  the model began to overestimate total C stocks. Model parameter estimates converged on an actual input range from 2.0 to 3.0, and a mean value of 2.5 was used as the final model input (Table 2). Modeled decay rates were fastest for pool 1 (free-light fraction) and slowest for pool 3 (dense fraction). The greatest proportionate transfers were from pool 1 to 2 (free to occlude light fractions) and 2 to 3 (occluded light to dense fractions), with lower flows in reverse directions.



**FIGURE 5** Transit time distribution and mean system ages of the three modeled carbon pools when the system experienced an input of  $2.5 \text{ Mg C ha}^{-1} \text{ year}^{-1}$

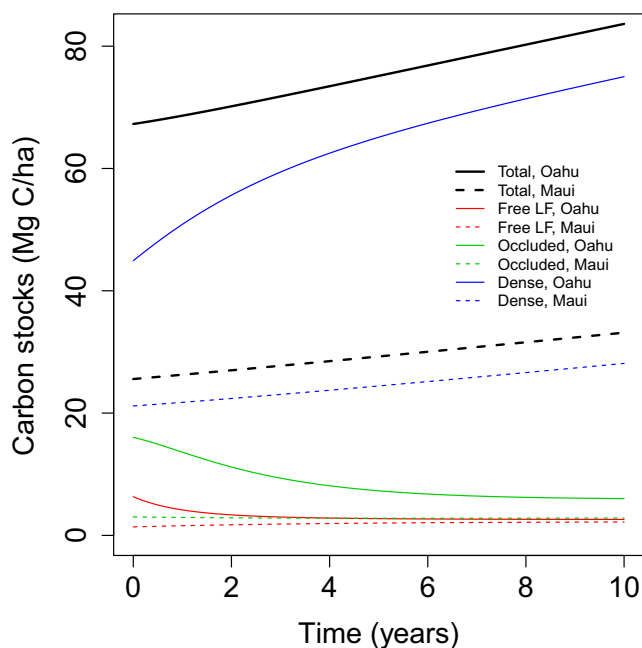
The transit time distribution obtained for the final model (Figure 5), shows that respired C from this system is initially dominated by C from the free-light fraction in the first 0–5 years, with older, but smaller, contributions from the occluded light and the dense fractions. Old C in the respiration

flux was dominated by C from the dense fraction. The transit time distribution is only for steady-state conditions once the system equilibrates. The mean transit time for C entering the soil system obtained for this model was 177 years, and the median of the transit time distribution (50% quantile) was 110 years (Table 3).

Comparing predictions from the energycane system measured on the island of Maui with the model derived for the crop system on the island of Oahu, similar accumulation trends of C over time were observed, but with different base levels of initial C (Figure 6). In particular, a similar trend (slope) of C accumulation occurred in the dense fraction.

**TABLE 3** Mean and median age and transit times with associated uncertainty when inputs were varied from 2 to 3 Mg C ha<sup>-1</sup> year<sup>-1</sup> by 0.05 increments

	Time (years)
Mean pool ages	
Free light fraction	28 ± 7
Occluded light fraction	112 ± 22
Dense fraction	210 ± 35
System age	
Mean	207 ± 35
Median	143 ± 24
Transit time	
Mean	177 ± 32
Median	110 ± 21



**FIGURE 6** Temporal evolution of total C stocks and density fractions for soils on Oahu and Maui

## 4 | DISCUSSION

### 4.1 | Ecosystem development and C inputs

Energycane is a high yielding bioenergy crop with potential for adoption as a biofuel feedstock in Hawaii (Morgan et al., 2019) that we found to also draw down substantial atmospheric C into stable mineral-associated organic matter pools when in no-till management. Crop canopies in ratoon systems typically develop earlier and faster than canopies of plant crop systems (Matsuoka, Kennedy, dos Santos, Tomazela, & Rubio, 2014; Matsuoka & Stolf, 2012), but the stalk portion left after each ratoon harvest results in a much greater number of buds to support future growth than the plant crop. Following harvest, the rapid redevelopment of regrowth is associated with a high fraction of incident radiation interception (Robertson, Wood, & Muchow, 1996).

Poorter et al. (2012) reported that 80%–85% of plant biomass in general is partitioned to aboveground leaves and stems with the remaining 15%–20% allocated to roots. In a study of annual tropical crops, the partitioning to roots ranged from 10% to 12% (Squire, 1990). In early studies, it was thought that the whole root system dies after the harvest of perennial grasses, and the growth following ratoon cycles depends on the formation of a new root system (Clements, 1980). More recently, it was found that the portion of the stem (stool) that remains alive after harvest in ratoon system, which in our case was 20 cm in height, will provide sufficient photosynthate for regeneration (Matsuoka & Stolf, 2012) and the old root system will retain some functionality (Smith, Inman-Bamber, & Thorburn, 2005).

The intact belowground physical and ecological community supports the rapid regrowth of aboveground biomass and the predominance of surface root (0–40 cm) distribution for energycane agreed with previous studies (Ball-Coelho et al., 1992; Evensen, Muchow, El-Swaify, & Osgood, 1997; Matsuoka et al., 2014; Pawlowski et al., 2017; Smith et al., 2005). The shallow rooting of the crops is consistent with other perennial grasses, but also with the use of buried drip system for irrigation and fertigation (injection of soluble fertilizer through the irrigation lines) at HC&S. This system provides an efficient, consistent supply of water and nutrients to the biofuel crops but may have reduced the need for an extensive deep root system (Pawlowski et al., 2017) and therefore root-derived C inputs that may have influenced accumulation.

### 4.2 | Soil C accumulation

Following over a century of intensive sugarcane cultivation, C accumulated rapidly when planted in a high yielding, no-till energycane system. As the crops were establishing





the root system and rhizosphere in the first 2 years, surface layer accumulation rates were high ( $4.0 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ). Similarly, deep soil C accumulation rates averaged  $22.2 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ , consistent with short-term gains in other tropical perennial grasses in an Oxisol at another field site at HC&S (Pawlowski et al., 2017, 2018) and in a Mollisol on Oahu, Hawaii (Sumiyoshi et al., 2016). Once the crop was established, soil C stocks continued to accrue, but also varied year to year in response to the environmental and growing conditions. The soil C accumulation rates over the 4 year study were lower and averaged 2.5 and  $11.4 \text{ C ha}^{-1} \text{ year}^{-1}$  for surface and deep soil, respectively.

Tropical ecosystems sustain high rates of plant inputs and soil organic matter turnover and can sequester up to twice as much soil C under no-till when compared to temperate environments (Six, Conant, Paul, & Paustian, 2002). On average, 46% of belowground C inputs were retained in soil organic matter in a range of agricultural field studies (Jackson et al., 2017). Perennial crops and low- or no-till systems with little residue removal are the most likely to result in a net increase or maintenance of soil organic C (Lewandowski, 2013). Crow et al. (2018) showed that C accrual in a similar study system on Oahu, Hawaii, was due to rapid transfer of fresh root-derived inputs through soil aggregates and into more stable mineral-dominated pools. Simulations of warmer conditions expected with climate change reduced the rate of C accumulation, but the effect of greater temperature on decomposition was not enough to overcome the effect of stabilization with minerals and net accumulation continued over time (Crow & Sierra, 2018).

Enhanced root activity in the rhizosphere can induce priming, or stimulation of soil C decomposition, which may result in a net loss of soil C (Blagodatskaya & Kuzyakov, 2008). The decrease in particulate organic debris in the light and occluded fraction suggests stimulated decomposition of microbially available C. However, instead of priming reducing soil C, we observed a transfer to mineral-associated organic matter pools. De Graaff, Jastrow, Gillette, Johns, and Wulschleger (2014) found switchgrass cultivars differentially regulating available C through the soil profile, which in turn affected priming. In that study, greater priming occurred in shallow soil compared to deeper depths, and increased root exudation only marginally affected decomposition at depth. We observed steady increases in measured C stocks from  $180.1 \pm 9.7$  in the baseline to  $225.7 \pm 7.0$  by Year 4 at deeper depths. Thus, although deep soil C losses from priming are of concern with increases in yield, it did not result in a net loss in our study.

### 4.3 | Energycane C dynamics and persistence

The data and subsequent modeling supported the hypothesis that soil C not only accumulates but will persist for long

periods of time in mineral-associated organic matter pools under no-till management of cultivated energycane. The method used to sample roots may have underestimated the total amount of root C inputs; however, measured root values cannot account for root turnover and flux occurring dynamically throughout the year. The measured root C in the surface soil ( $0.59 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ) and the fraction model's required input ( $>2.0 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ), when taken together, indicate that yearly measurement of root biomass C in the surface soil likely accounted for only 25% of inputs expected by the fraction model. This broadly indicates that root turnover and exudation account for as much as 75% of C input, which is especially important when considering that this system is accumulating rapidly degraded carbon in the dense mineral-rich soil fraction. Better understanding of root dynamics and further identification of the exact mechanism of accumulation in the dense fraction will be important future research to predict and control C sequestration in this and similar tropical soil systems.

From the model results, we inferred that the accumulation occurred mostly due to transfers of photosynthetically fixed C that entered the system through the free-light fraction and rapidly transferred to the dense fraction. The Bayesian optimization procedure provides posterior parameter uncertainties to compute the uncertainty in predictions. This uncertainty increased with simulation time, indicating that the range of expected responses of total C stocks increases with time and is mainly dominated by uncertainty of the response of the mineral fraction.

A predicted mean transit time of 177 years, with a median of 110 years, suggests that very old C from the dense fraction contributed to losses from the system, such as respiration, and that fresh C from roots was not rapidly decomposing. The transit time distribution can also be interpreted in terms of the fate of a unit of C that enters the soil and leaves the system in subsequent times. Our fitted model suggests that first, a significant proportion of the C would be released early from the free-light fraction. With a time lag of a few years, a portion of the C transferred to the occluded light fraction gets respired and transferred to the dense fraction. In the dense fraction, where we expect mineral stabilization and long-term storage, this C starts contributing to the release flux in later years.

There is increasing evidence that the mean transit time in ecosystems and soils is on the order of decades to centuries (Lu, Wang, Luo, & Jiang, 2018; Sierra et al., 2018). In particular, tropical ecosystems seem to have transit times in the order of decades, while temperate and boreal ecosystems can have transit times in the order of centuries. Carbon stabilization in mineral surfaces can significantly increase the transit time of C in soils (Rasmussen et al., 2018; Sierra et al., 2018), and since a relatively large proportion of C is transferred from fast to slow cycling pools in the studied ecosystem, the obtained mean transit time for this system is relatively long compared to other tropical sites where mean transit times

may range between 10 and 35 years (Lu et al., 2018). The long transit times predicted for this perennial grass system can be considered as an additional benefit of C sequestration since not only more C gets stored in the system, but it is also retained there for a relatively long time.

#### 4.4 | Carbon flow through multiple systems

Comparing predictions with the empirical model developed for energycane at HC&S commercial field 718 to other systems is a critical step to assessing the potential to apply the modeling framework across similar systems. In a similar field trial conducted at the Waimanalo Experiment Station on the island of Oahu, changes in soil C pools were quantified, modeled, and projected to understand the belowground dynamics following cultivation of grassland in napiergrass. The soils at both sites were Mollisols (of different series) and both studies cultivated perennial grasses (although different species) in a no-till ratoon harvest system. Unlike at HC&S on Maui, the soils at Waimanalo were not previously intensively cultivated. At least for the last 24 years, the field site was maintained as a grassy area among other test fields, which resulted in the different base levels of initial C. Importantly, a similar C accumulation rate, driven by increases in the dense (i.e., mineral-dominated) fraction, further supports the hypothesis that C is quickly transferred from the free-light to the dense fraction and strengthens the argument that C transferred to the soil is likely to be sequestered in tropical perennial grass systems.

## 5 | CONCLUSION

Without understanding the amount and fate of C inputs to the belowground system, it is not possible to fully quantify the potential climate benefits of productive tropical perennial grass agroecosystems. Energycane emerged as a strong candidate for soil C sequestration due to allocation of resources to roots and accumulation of soil C that persists for over a century even in the surface soil layers. Model results demonstrate consistent accumulation of C in the mineral-associated organic matter of soils in similar tropical perennial grass systems despite differences in species and direct root inputs. These models can form the basis for tools to help project the full potential climate benefits of bioenergy production by allowing the inclusion of soil C in system-level C budgets.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information of this article.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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